Naval Health Research Center Detachment (Toxicology)

DESIGN OF A PLETHYSMOGRAPH FOR THE MEASUREMENT OF PULMONARY MECHANICS AND INTRAPLEURAL PRESSURE IN SMALL ANIMALS DURING EXPOSURE WITHOUT SURGICAL INTERVENTION

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PREFACE

This is an interim report describing part of the research efforts at the Naval Health Research Center Detachment Toxicology NHRC/TD) to assess the risk for the development of acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) from inhalation of smoke and other airborne toxicants of military interest. This report specifically describes the design and use of a novel device to measure respiratory mechanics in small animals during exposure to airborne toxins. The combined plethysmograph/exposure tube (PET) described herein was developed to measure acute lung responses to exposure in an advantageous, precision manner heretofore not possible with existing devices of a similar nature. This work was sponsored by the Naval Medical Research and Development Command under Work Unit # 63706N-M00095.004.1714 and was performed under the direction of CAPT Kenneth R. Still, MSC, USN, Officer-in-Charge NHRC/TD.

The opinions contained herein are those of the author and are not to be construed as official or reflecting the view of the Department of the Navy or the Naval Services at large. A patent application has been submitted.

Animal handling procedures depicted in this presentation were subject to review and approval by the Animal Care and Use Committee located at Wright-Patterson AFB and the Office of Air Force Surgeon General. The animals shown in this presentation were fully anesthetized. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Research, National Research Council, DHHS, National Institutes of Health. Publication 85-23, 1985 and the Animal Welfare Act of 1966, as amended.

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EXECUTIVE SUMMARY

PROBLEM

Among the variety of pulmonary responses, which can be elicited by inhaled agents/contaminants of military interest are those that are acute in onset and can be immediately lethal. Pulmonary responses of this nature are bronchoconstriction, bronchospasm, general respiratory irritation, and respiratory sensitization or hyper-reactivity (AHR), which often are collectively known as airway reactivity (AR) responses. Many AR responses, with exception of AHR, manifest themselves only during or shortly after exposure. Because AR responses are not necessarily founded in tissue damage their onset, severity, and progression often can only characterized using physiological means. Physiological testing (also known as pulmonary function testing or pfts) may be the only means to evaluate the potential of an airborne chemical to elicit an untoward pulmonary effect in a small animal test subject. The best manner to evaluate an AR response directly is through the measurement of the mechanical properties of the lung (elastic, resistive, and inertial properties) which are associated breathing. Classic measures of pulmonary mechanics are lung resistance (R_L) and dynamic compliance (C_{dyn}). Calculation of both of these functional parameters requires the measurement of pleural pressure (Ppl), which is the driving force of breathing. Heretofore, methods used to measure Ppl and therefore R_L and C_{dyn} in a small animal during exposure has required surgical intervention. The need for surgical intervention causes technological problems, which limit experimental protocol as well as theoretical problems with the interpretation of experimental results. To avoid the necessity for surgical intervention, indirect measures of AR have been developed. Unfortunately, these indirect measures are predicated on poorly defined assumptions and suffer from lack of precision and high variability in "normal" subjects. Therefore data interpretation of experimental results is complicated further. Consequently the measurement of AR responses in a dosimetric manner, which is essential for risk assessment, is subject to a wide margin of error using existing methods.

OBJECTIVE

The objective of this work was to build a device (combined plethysmograph/exposure tube or PET) that expanded on an earlier discovery and could be used to measure R_L and C_{dyn} during exposure without the need for surgical intervention.

APPROACH

The approach was to incorporate into an existing improved head-out plethysmograph (also a recent invention of this laboratory) an esophageal catheter for measurement of esophageal pressure (Pes), which is an well-accepted and highly accurate estimator of Ppl. A primary design criteria for the device was obtain a reliable measurement of Ppl without the need for animal surgery and without the need for unnecessary penetrations of an exposure chamber for either pressure transducer leads or to connect the esophageal catheter to its transducer. A comparison among measurements of ventilation, breath structure, and pulmonary mechanics made with the PET and more conventional plethysmographic methods was conducted to determine if use of the PET would result measurement artifact.

RESULTS

The design of the PET fulfills the objectives stated as shown both by the data and by the consensus approval from other recognized experts in the field when presented at a national scientific meeting (Society of Toxicology, March 1999).

CONCLUSION

The PET is a successful device that will bring a new level of technological accuracy and scientific relevance into the measurement AR type pulmonary responses elicited during exposure of experimental animals. This may well lead to improved dosimetric evaluation of the potential for inhaled agents/contaminants to elicit untoward respiratory effects that could compromise personnel performance, health, and mortality.

ABSTRACT

The real-time measurement of changes in respiratory mechanics, primarily dynamic compliance (C_{dyn}) and airway resistance (R_L), is often used to assess the pulmonary toxicity of inhaled materials and irritants thought to elicit an airway reactivity response. A simple volume displacement plethysmograph used for measurement of ventilation in spontaneously breathing rats was modified for the determination of C_{dyn} and R_L by including measurement of intrapleural pressure (Ppl). Accurate estimates of Ppl were obtained by measurement of esophageal pressure (Pes) using trans-oral insertion of a water filled catheter. Measurement of Pes did not require surgical intervention as is often required for measurement of Ppl directly. The use of conventional head-out plethysmography to measure ventilation and respiratory mechanics during exposure usually precludes the use of trans-oral insertion of an esophageal catheter to measure Pes. Thus, invasive methods must be used to measure Ppl. The combination head-out plethysmograph/nose-only exposure tube (PET), presently described, was found suitable for measurement of R_L and C_{dyn} using trans-oral catheterization for determination of Pes during exposure. Use of PET required did not require surgical intervention, did not obstruct the animal's normal breathing, and did not require extraordinary procedures for connection to a nose-only exposure chamber. Ventilation, breath waveform, and respiratory mechanics measurements in 36 Long Evans rats demonstrated that neither short-term restraint in the PET nor subsequent insertion of the esophageal catheter significantly altered ventilation or individual breath structure. R_L and C_{dyn} measured in normal rats using the PET did not differ from R_L and C_{dyn} determined using more conventional plethysmographic methods.

KEY WORDS

Non-invasive, intrapleural pressure, resistance, compliance, airway reactivity, plethysmography

LIST OF ABBREVIATIONS

Note common chemical and measurement abbreviations are not included.

R_L	lung resistance
C_{dyn}	dynamic compliance
Ppl	intrapleural pressure
Pes	esophageal pressure
PET	plethysmograph/exposure tube
Pfts	pulmonary function tests
AR	airway reactivity
Paw	pressure at airway opening
Ptp	transpulmonary pressure
i.p.	intraperitoneal
Penh	enhanced pause
FDP	flow derived parameter
PIF	peak inspiratory flow
PEF	peak expiratory flow
Vt	tidal volume
f	breathing frequency
Ve	minute ventilation
Ti	inspiratory time
Te	expiratory time
Rt	relaxation time

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INTRODUCTION

Pulmonary function tests (pfts) are useful, relatively nondestructive tools for the evaluation of the effects of inhaled toxins (O'Neil and Raub,1984, Mauderly, 1989). Because function is closely coupled with structure, pfts can be used to determine the presence, nature, and extent of damage to lung tissue (Bates et al., 1971). However, not all toxic responses of the lung are necessarily a manifestation of tissue damage. In these cases pfts may be singular or best means to characterize an untoward pulmonary response (Silbaugh, et al., 1981). Pfts when used in conjunction with other assays provide information that correlate early chemical biomarkers of susceptibility, effect, and prognosis with clinically significant pulmonary responses. Pfts often provide information about the progression of lung disease and can be used as indices of disability.

A variety of pfts have been developed in small animals that describe aspects of breathing and lung function; such as ventilation, distribution of ventilation, the mechanics of breathing (dynamic and static - elastic, inertial, and resistive properties), reserve capacity, and gas exchange. Although functional changes are implicit of structural changes they are not explicit descriptors of those changes. Nevertheless, patterns of functional change can be definitive of certain categories of lung disease (Drazen, 1976, Kimmel et al., 1985), and collective interpretation of several pfts provides the most accurate functional characterization of given types of pulmonary injury in toxicity studies (Costa and Tepper, 1988). Many small animal pfts were derived from human procedures. The functional response in animals and humans to different types of lung injury/disease is very similar (Mauderly,1988). Thus the use of small animal pfts to assess the potential impact of an inhaled toxin on humans is well founded.

As noted above, assays of function may be the sole effective means to characterize an untoward response to an inhaled toxin. These responses can be elicited without permanent underlying structural alteration and may be transient in nature. Bronchoconstriction/spasm and changes of airway reactivity (AR) are responses of this type. Often they are elicited during exposure, and may subside shortly after cessation of the exposure. Measurements of changes in ventilation and breath structure are often used to characterize these types of responses (Hamelman et al., 1997, Pauluhn, 1997). These measurements can be made during exposures in

unrestricted animals using barometric plethysmogaphy (Dorobaugh and Fenn, 1955, Pennock et al., 1979). However, some of the principles and assumptions underlying barometric techniques limit its usefulness as a tool for functional assay in toxicological studies (Epstein and Epstein, 1978). Since the pioneering work of Amdur and Mead (1958) and numerous others, the measurement the dynamic mechanical properties, dynamic compliance (Cdyn) and airway (or lung) resistance (R_L), is considered a more direct method to quantitate AR type responses (Drazen, 1983, O'Neil and Raub, 1984). However, calculation of both Cdyn and R_L require the measurement of transpulmonary pressure (Ptp) which is the driving force of breathing and is determined as the difference between pressure at the airway opening (Paw) and pressure in the pleural space, intrapleural pressure (Ppl). The method of Amdur and Mead uses head-out, volume displacement plethysmography, in which the animal's head or nose is outside of the plethysmograph body. This facilitates placement of the animal's head/nose directly in the airstream under investigation. Surgical insertion of a fluid filled catheter through the chest wall into the pleural space is used to obtain Ppl. Placement of this catheter is a difficult procedure often resulting in damage to the lung tissue. Exteriorizing the catheter through the plethysmograph wall for connection to a pressure transducer is technologically difficult. In all it is difficult to keep the catheter patent, particularly when repeated measurements are desired. Consequently, other investigators have adopted the less difficult and less invasive method of trans-oral insertion of a fluid filled esophageal catheter to obtain esophageal pressure (Pes), a valid estimate of Ppl (Davidson et al., 1966, Palecek, 1968). This technique is used in preparations where the whole animal is enclosed in the plethysmograph and breathing takes place through an exteriorized tracheal cannula (Diamond, and O'Donnell, 1977). However, bypass of the extrathoracic airways is not relevant to most inhalation toxicity studies in which assessment of pfts during exposure is desired. In a head out plethysmograph trans-oral placement of an esophageal catheter leads to an obstruction that makes placement of the animal's head or nose into an exposure chamber difficult. One possible solution would be to place the entire plethysmograph into the exposure chamber. This is inconvenient, would necessitate passing transducer leads through chamber walls for connection to signal amplifiers and recorders, and the exposure atmosphere may be damaging to plethysmograph parts. Using a head or nose out plethysmograph with an esophageal catheter with a nose-only exposure system would require exteriorization of the catheter through the chamber wall at minimum.

A combination plethysmograph/exposure tube (PET) has been designed which:

- permits the use of an esophageal catheter to obtain Pes an estimate of Ppl.
- poses little obstruction to breathing.
- does not require penetration of the exposure chamber walls.
- can be used with a nose-only exposure chamber in a manner no different than conventional exposure tubes.
- is suitable for measurement of ventilation, breath structure and dynamic pulmonary mechanics in small animals during exposure. Hence can be used to evaluate AR effects elicited by inhaled agents.

METHODS AND MATERIALS

PET CONSTRUCTION

The PET is a modified head-out plethysmograph and was fabricated, in house, from Plexiglass® bar and tube stock (Figures 1,2) for an estimated cost of $750.00 per unit, materials and labor. The PET shown has a 350 g animal capacity. It is most commonly used as a volume displacement plethysmograph (flow box), however it can be configured for constant volume (pressure box) operation. The PET consists of 4 separate pieces, which fit together with o-ring seals. They are the nose, thorax, body, and tailpieces. The dimensions (inches) for each of the pieces are given in Figure 3.

The internal taper of the nose-piece conforms to the shape of the snout of a laboratory rat, so that the animal's nose protrudes to the end of the breathing port opening with minimal contact between the animal's eyes and the nose-piece wall. The nosepiece has a concentric internal cylinder leading to the tapered portion (Figure 4). A latex (dental dam) membrane with a small opening cut in the center is stretched over the rear (non-tapered end) of the cylinder and is held in place with an o-ring and a wide elastic band. This latex membrane serves as the neck seal between the exterior and interior of the plethysmograph (Figure 5). The nosepiece of the PET shown is sized to fit into the ports on a Cannon nose-only exposure chamber (Cannon et al., 1983). A 38 cm French #5 infant feeding tube (0.17 cm o.d., Professional Medical Products, Inc.,

Greenwood, SC) is used for the esophageal catheter (Figure 4,5). One end of the catheter has a luer fitting for connection to a pressure transducer (SX01DN 0 – 1 psi, SynSym Inc., Malpitas, CA) for measurement of Pes. This connection is located on the exterior front of the nose-piece. The catheter penetrates the front of the nosepiece through the outer cylinder and then is looped to penetrate the inner cylinder. This positioning of the catheter permits trans-oral insertion of the catheter into the esophagus without obstructing connection of the PET to a nose-only exposure chamber port. For Pes measurements the transducer and catheter are water filled and cleared of bubbles. All of the penetrations of the nose-piece are sealed with silastic (Figure 4-6).

The thorax-piece is configured with two internal diameters. The forward diameter (toward the nosepiece) is the slightly larger of the two. The internal ridge created by this transition forms a restraint that fits behind the animal's forepaws and inhibits withdrawal of the animal's upper body and nose away from the breathing port opening. Attached to the thorax piece is a holder that assures Pes transducer placement at animal chest height.

The body-piece of the PET houses the animal's abdomen and "lower" body and is fitted with 1-cm diameter pneumotachygraph and flow (volume) transducer (MP45-14, Validyne Engineering, Northridge, CA) ports. The pneumotachygraph assembly consists of a ¾ " fnpt fitting attached to the PET wall. This fitting accepts a ¾ " mnpt plug with a 1-cm diameter bore hole. This plug, with an o-ring seal, holds 6 layers of ¾ " diameter #325 mesh stainless steel wire cloth in place over the pneumotachygraph port. The transducer and pneumotachygraph ports are located directly opposite one another in the body-piece walls. The body-piece of the version of the PET shown has a sidearm, which is plugged with a plunger assembly. Positioning of plunger in this sidearm can be used to alter PET volume, if needed, when the PET is configured as pressure plethysmograph. This sidearm also can be connected to a large volume reservoir containing heat-adsorbing material to adjust PET performance during pressure box operation.

The tailpiece of the PET has a locking, bulkhead fitting through which a ¼ " diameter rod passes. The interior end of the rod has a flat plate attached, and the whole assembly can be adjusted to serve as a stop to restrict movement of the animal within the PET. The tailpiece also is fitted with a luer port for exteriorization of an in-dwelling blood sampling catheter.

PULMONARY FUNCTION TESTING

Animals

Thirty-six, female Long-Evans rats (Charles River Labs, Raleigh, NC) weighing 242 ± 18 g were used for this study. The animals were housed over absorbent bedding in plastic shoebox type cages, and were fed and watered *ad libitum*. Animals undergoing PET plethysmography were lightly anesthetized with urethan (ethyl carbamate, as needed, approximately 0.5 g/kg – i.p.) to facilitate loading into the PET. To simulate PET conditions and to minimize artifact from animal exploration, animals undergoing barometric plethysmography also were lightly anesthetized. Animals undergoing Diamond box (see below) plethysmography received three times this dose to induce surgical depth anesthesia.

Plethysmography

Performance of the PET, operated as a flow box, was assessed by comparing ventilation, breath structure (flow-derived parameters), and dynamic mechanics measurements to those derived from other well characterized, plethysmographic techniques. See Table 1 for definitions and units of the parameters measured. A 4.5 L barometric plethysmograph (Fenn box –PLY3115, Buxco Electronics, Sharon, CT) was used to measure ventilation and flow-derived parameters in unrestrained animals. Flow through the pneumotachygraph on the Fenn box was measured using a differential pressure transducer (SOX1, 0 - 1 psi, SynSym Inc., Malpitas CA). These data were compared to those derived from the PET without the use of the esophageal catheter. Although the underlying principles of measurement between barometric and volume displacement plethysmography differ, it was thought that this comparison would serve as an indicator of the effects of restraint in the PET on ventilation and breath structure. A comparison was made between ventilation and flow-derived parameter measurements made with and without the use of the esophageal catheter to assess the effects of catheter insertion on breathing. The transducer signals were processed using Buxco Electronics Max II hardware. The acquired data were analyzed using Buxco Electronics, Biosystems XA software. Indices of the dynamic mechanical properties of the lung, R_L and C_{dyn}, derived from the PET were compared to those taken in

animals using a well established plethysmographic technique for this purpose (Diamond and O'Donnell, 1977; Kimmel and Diamond, 1984). These latter measurements were made using a 1.5 L whole-body plethysmograph (Diamond box – PLY 3114, Buxco Electronics, Sharon, CT). Pes and flow transducers were the same as described for the PET. The signal processing hardware and data analysis software described above was used. In this preparation the animals were tracheotomized, and breathed through a port in the plethysmograph wall. Combined tracheal cannula and breathing port dead space was 1.1 ml. The reader is referred to Diamond and O'Donnell (1977), Sabo and colleagues (1984) or Kimmel and Diamond (1984) for a general description of the methods used. Regardless of plethysmographic technique, each animal underwent testing for 10 to 15 minutes with a minimum of 30 breaths per minute being analyzed.

Statistics

Data were subject to multiple Student's-t test for comparison of means. Multiple plethysmographic techniques (except Diamond box plethysmography) were applied to 5 animals. These data were analyzed using paired t-tests However, the data presented are those from non-paired t-tests, the results of which did not differ from the paired t-tests.

RESULTS

ASSEMBLY AND LOADING OF THE PET

Various stages of loading and assembly of the PET are shown in Figures 7 – 11. The nosepiece with attached latex membrane (dental dam – medium density) is shown in Figure 5. A hole, slightly larger than 1-cm diameter, cut into the dental dam will allow insertion of the animal's head past the ears (Figure 7). The membrane alone provides a sufficient seal not to alter a calibration flow of 20 ml/sec passed through an assembled PET. Thus, no additional sealant or grease is required nor is it necessary to shave the animal's neck. Caution must exercised when stretching the dental dam over the internal cylinder of the nosepiece to prevent tearing of the dam material. The dam is secured with an o-ring and a wide elastic band. Using this method, it is not necessary to support the dental dam with additional material or metal stiffeners. The infant

feeding tube catheter is flexible enough to be inserted into the esophagus with ease and as shown in Figure 8 does not present a significant obstruction to the animals breathing zone. The exposed portion of the catheter can be shielded using a small wire spring to prevent the animal from chewing on the catheter. Likewise the exposed portion catheter can be coated, if necessary, to minimize absorption of test material from the exposure chamber.

As noted above, the thorax-piece has two internal diameters. The front portion being slightly larger that the rear portion; the animal placed head first from the rear of the thorax-piece far enough for the forepaws to clear the ridge formed by the transition between the two internal diameters (Figure 9). When completely assembled this ridge limits animal withdrawal from the front of the PET. Once loaded into the thorax-piece the animal's head can be placed into the nosepiece and the two pieces connected (Figure 10). The resulting assembly can be slid into the body-piece (Figure 11). There is approximately 8 cm of adjustment space in the overall assembly to accommodate different size animals. Additional restraint to animal motion within the PET is provided by adjustment of the restraining rod assembly in the tailpiece (not shown).

PULMONARY FUNCTION TESTS

Measures of ventilation, flow-derived parameters, and lung dynamic mechanical properties collected using the Fenn box, Diamond box, and PET (with and without esophageal catheter) plethysmography are shown in Table 2. There were no significant differences among ventilatory or flow-derived parameters as measured by Fenn box, PET with, and PET without esophageal catheter plethysmography. PEF, PIF and the non-dimensional parameters Penh and FDP were significantly greater in animals undergoing Diamond box plethysmography. This is most likely due an increased Ppl (not shown) generated by these animals to overcome the added 1.2 ml dead space contributed by the tracheal cannula and the valve assembly (for collection other pfts) on the plethysmograph breathing port. This increased Ppl led to significantly greater flows and an elevated tidal volume. Despite differences between flows and flow-derived parameters, direct measures of lung dynamic mechanical properties, C_{dyn} and R_L, were not different between PET and Diamond box techniques.

DISCUSSION/CONCLUSION

Numerous non-invasive, plethysmograpic methods have been developed to assess AR in small animals (reviewed - Costa and Tepper, 1988; Mauderly, 1989). Most are an attempt to avoid the use of either pleural or esophageal catheters to measure Ppl. Pleural catheterization is invasive and esophageal catheterization, though relatively non-invasive, poses technical difficulties when applied to pulmonary function measurements real-time, during inhalation exposure. Consequently, both barometric and head-out plethysmographic techniques have been used extensively to examine AR reponse to inhaled toxins and pharmaceuticals. Barometric methods rely upon indirect measures of ventilation as well as an examination of breath structure, in the form of calculated flow-derived parameters, to characterize AR responses. Although in popular use, numerous investigators have questioned the accuracy and sensitivity of barometric methods for measurement of flow and volume in all but ideal conditions and animal status. Other pulmonary responses such as gas trapping can interfere with barometric measurement techniques to assess AR responses (Silbaugh et al., 1981). Head-out plethysmography (either flow or pressure) provides a more direct assessment of ventilation, hence flow-derived parameters. The PET described herein provides ventilation and flow-derived parameter data comparable to that from barometric and similar head-out devices. Nevertheless, changes in ventilation and breath structure are the result of many factors, of which is airway tone and AR responses are only a part. Although influenced by AR, changes in ventilation and flow-derived parameters are themselves indirect measures of AR responses.

Lung dynamic mechanical properties, C_{dyn} and R_L, are measures of the elastic and resistive forces associated with breathing and airway condition, hence AR response. Examination of these parameters provides a more direct assessment of AR responses, bypassing some of the vagaries associated with reliance upon ventilation and flow-derived parameters that are gathered by fundamentally barometric means to assess AR response. The PET can be readily used to determine C_{dyn} and R_L during exposure without the difficulties associated with surgical implantation of a pleural catheter or many of the technological difficulties associated conventional use of an esophageal catheter in conjunction with an exposure chamber.

Recently, Pauluhn (1997) reviewed the guinea pig model of respiratory hypersensitivity and reported on a method to refine analysis of ventilation and flow-derived data in order to develop a more objective assessment of AR response. Much of the variability and inconsistency in these assessments of AR, including false positive responses, could be attributed to ill-defined factors in barometric and head-out plethysmographic methodology. The PET shown was fabricated for use with rats, which have had limited use in AR studies. We are presently developing a version of the PET suitable for use with guinea pigs, which have become the standard model for AR work. The PET may be a viable alternative to other plethysmographic methods for assessing AR responses to inhaled toxins or pharmaceuticals, particularly during inhalation exposure.

REFERENCES

Amdur, M.O. and Mead, J. 1958. Mechanics of respiration in unanesthetized guinea pigs. *Am. J Physiol.* 102(2):364-368.

Bates, D.V., Macklem, P.T., and Christie, R.V. 1971. *Respiratory Function in Disease.* WB Saunders, Phildelphia, PA.

Cannon, W.C., Blanton, E.F., and McDonald, K.E. 1983. The flow past chamber: An improved nose-only exposure system for rodents. *Am. Ind. Hyg. Assoc. J.* 44:923-928.

Costa, D.L. and Tepper, J.S. 1988. Approaches to lung function assessment in small animals. In: *Toxicology of the Lung.* Gardner, D.E., Crapo, J.D., and Massaro, E.J. (eds). pp. 423-434. Raven Press, New York, NY.

Davidson, J.T., Wasserman, K., Lillington, G.A., and Schmidt, R.W. 1966. Pulmonary function testing in rabbits. *J. Appl. Physiol.* 21:1094-1098.

Diamond, L. and O'Donnell, M. 1977. Pulmonary mechanics in normal rats. *J. Appl. Physiol.: Respir. Environ. Exercise Physiol.* 43(6):942-948.

Drazen, J.M. 1976. Physiologic basis and interpretation of common indices of respiratory mechanical function. *Environ. Health Perspect.* 16:11-16.

Drazen, J.M. 1984. Physiological basis and interpretation of pulmonary mechanics. *Environ. Health Perspect.* 56:3-9.

Drorbaugh, J.E. and Fenn, W.O. 1955. A barometric method for measuring ventilation in infants. *J. Appl. Physiol.* 15:1069-1072.

Epstein, M.A.F., and Epstein, R.A. 1978. A theoretical analysis of the barometric method for measurement of tidal volume. *Respir. Physiol.* 32:105-120.

Hamelmann, E., Schwarze J., Takeda, K., Oshiba, A., Larsen, G.L., Irvin, C.G., and Gelfand, E.W. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* 156:766-775.

Kimmel, E.C. and Diamond, L. 1984. The role of nicotine in the pathogenesis of pulmonary emphysema. *Am. Rev. Resir. Dis.* 129:112-117.

Kimmel, E.C., Winsett, D.W., and Diamond, L. 1985. The augmentation of elastase-induced emphysema by cigarette smoke: Description of a model and review of possible mechanisms. *Am. Rev. Respir. Dis.* 132:885-894.

Mauderly, J.L. 1988. Comparison of respiratory function responses of laboratory animals and humans. In: *Inhalation Toxicology: The Design and Interpretation of Inhalation Studies and their Use in Risk Assessment.* U Mohr (ed). pp243-262. Springer Verlag, Inc. New York, NY.

Mauderly, J.L. 1989. Effect of Inhaled Toxicants on Pulmonary Function. In: *Concepts in Inhalation Toxicology,* McClellan, R.O. and Henderson, R.F. (eds). pp. 347 - 401. Hemisphere Publishing Co. New York, NY.

O'Neil, J.J., and Raub, J.A. 1984. Pulmonary function testing in small laboratory animals. *Environ. Health Perspect.* 56:11-22.

Palecek, F. 1969. Measurement of ventilatory mechanics in the rat. *J. Appl. Physiol.* 27(1):149-156.

Pauluhn, J. 1997. Assessment of respiratory hypersensitivity in guinea pigs sensitized to toluene diisocyanate: Improvements on analysis of respiratory response. *Fundam. Appl. Toxicol.* 40:211-219.

Pennock, B.E., Cox, C.P., Rogers, R.M., Cain, W.A., and Wells, J.H. 1979. A noninvasive technique for measurement of changes of specific airway resistance. *J. Appl. Physiol.* 46:399-406.

Sabo, J., Kimmel, E.C., and Diamond, L. 1984. The effects of the clara cell toxin 4-ipomeanol on pulmonary function in rats. *J. Appl. Physiol.* 54:337-344.

Silbaugh, S.A., Mauderly, J.L., and Macken, C.A. 1981. Effects of sulfuric acid and nitrogen dioxide on airway responsiveness of the guinea pig. *J. Toxicol. Enviro. Health* 8:31-45.

TABLE 1. Glossary of parameters and units

term	definition	units
Vt	tidal volume	ml
f	breathing frequency	breaths/min
Ve	minute ventilation	ml/min
Ti	inspiratory time	sec
Te	expiratory time	sec
Rt	relaxation time	sec
PEF	peak expiratory flow	ml/sec
PIF	peak inspiratory flow	ml/sec
Penh	enhanced pause (Te/Rt –1) (PEF/PIF)	nd*
FDP	flow-derived non-dimensional parameter PEF x (Te + Ti)/Vt	nd*
$\mathbf{C_{dyn}}$	dynamic compliance Vt/dPpl**	ml/cm H_20
$\mathbf{R_L}$	lung resistance dPpl/dflow***	cm H_20/ml/sec

* non-dimensional.

** difference in pleural pressure at points of zero flow.

*** difference in pleural pressure divided by absolute value of the difference between inspiratory and expiratory flow at points of equal volume.

TABLE 2. Comparison of ventilation and mechanics derived by different plethysmographic methods

pft	Fenn Box n = 9	PET w/o catheter n = 12	PET with catheter n = 10	Diamond Box n = 11
BodyWeight	233±10.2	234±12.9	238±15.2	255±21
Vt	1.10±0.21	1.36±0.15	1.44±0.23	1.61±0.34
f	128±29	101±20	87±15	104±32
Ve	129±27	135±21	125±20	167±41
Ti	0.22±0.05	0.28±0.07	0.30±0.02	0.25±0.04
Te	0.31±0.10	0.34±0.06	0.40±0.08	0.31±0.07
Rt	0.19±0.05	0.21±0.05	0.25±0.09	0.11±0.02
PEF	6.02±1.35	6.75±1.28	5.72±0.97	16.3±2.00*
PIF	7.65±1.37	7.35±1.65	6.85±1.17	11.4±1.83*
Penh	0.59±0.31	0.63±0.29	0.60±0.42	3.42±1.45*
FDP	2.84±0.40	3.06±0.44	2.81±0.47	5.19±1.00*
C_{dyn}	n/a	n/a	0.57±0.10	0.42±0.11
R_L	n/a	n/a	0.30±0.08	0.19±0.05

All values are mean ± standard deviation

* significantly different from all other methods at $p \leq 0.05$.

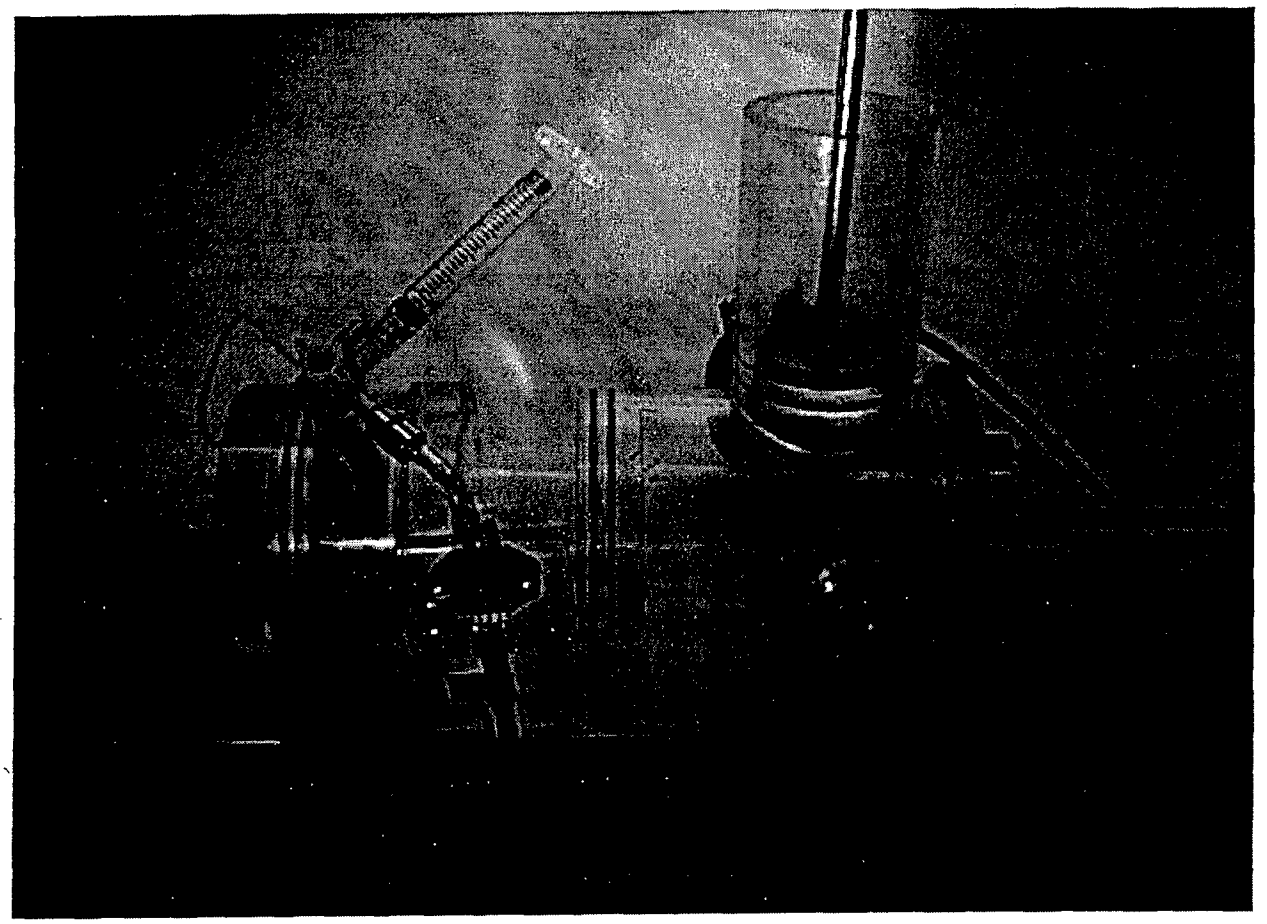

Figure 1. The fully assembled PET.

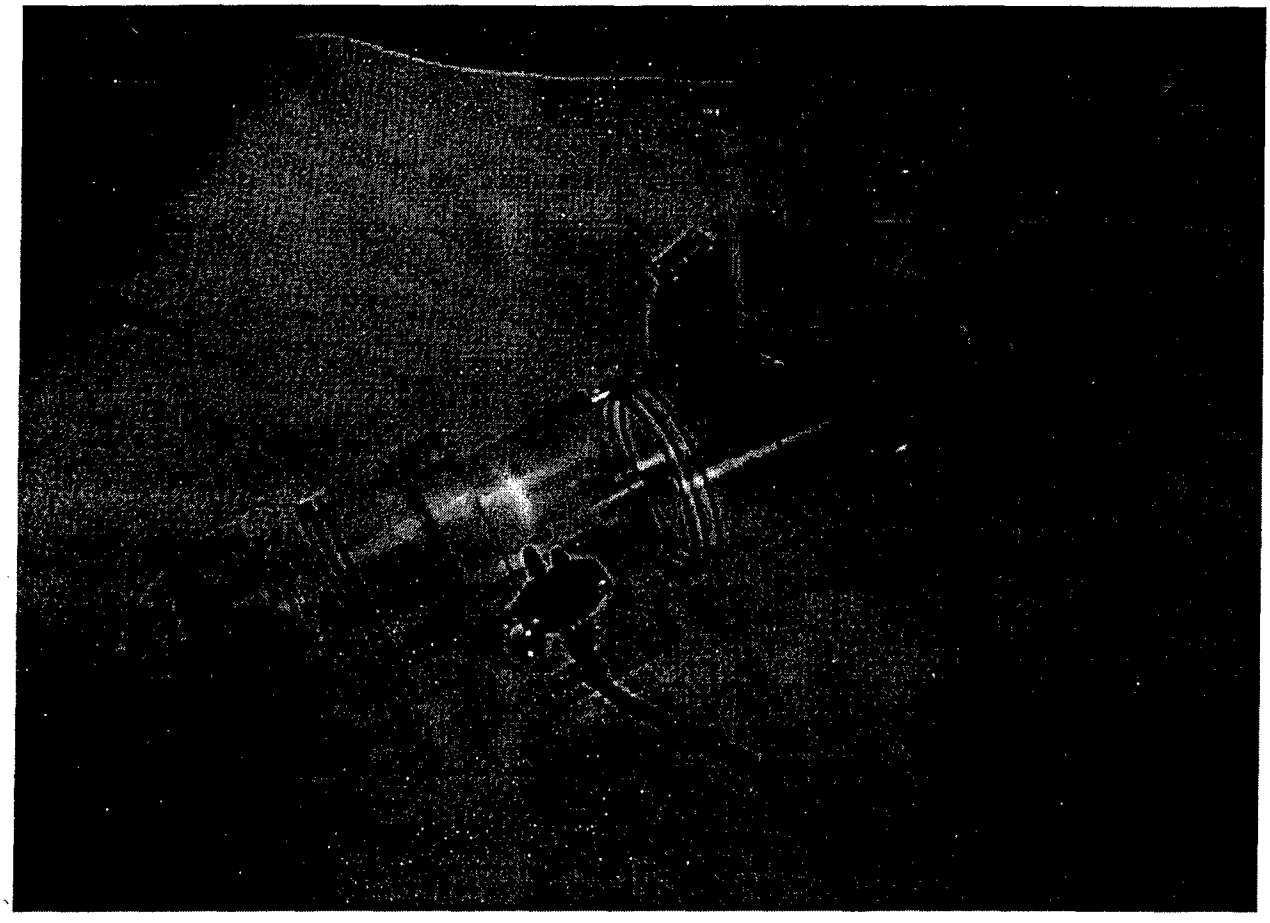

Figure 2. A disassembled PET.

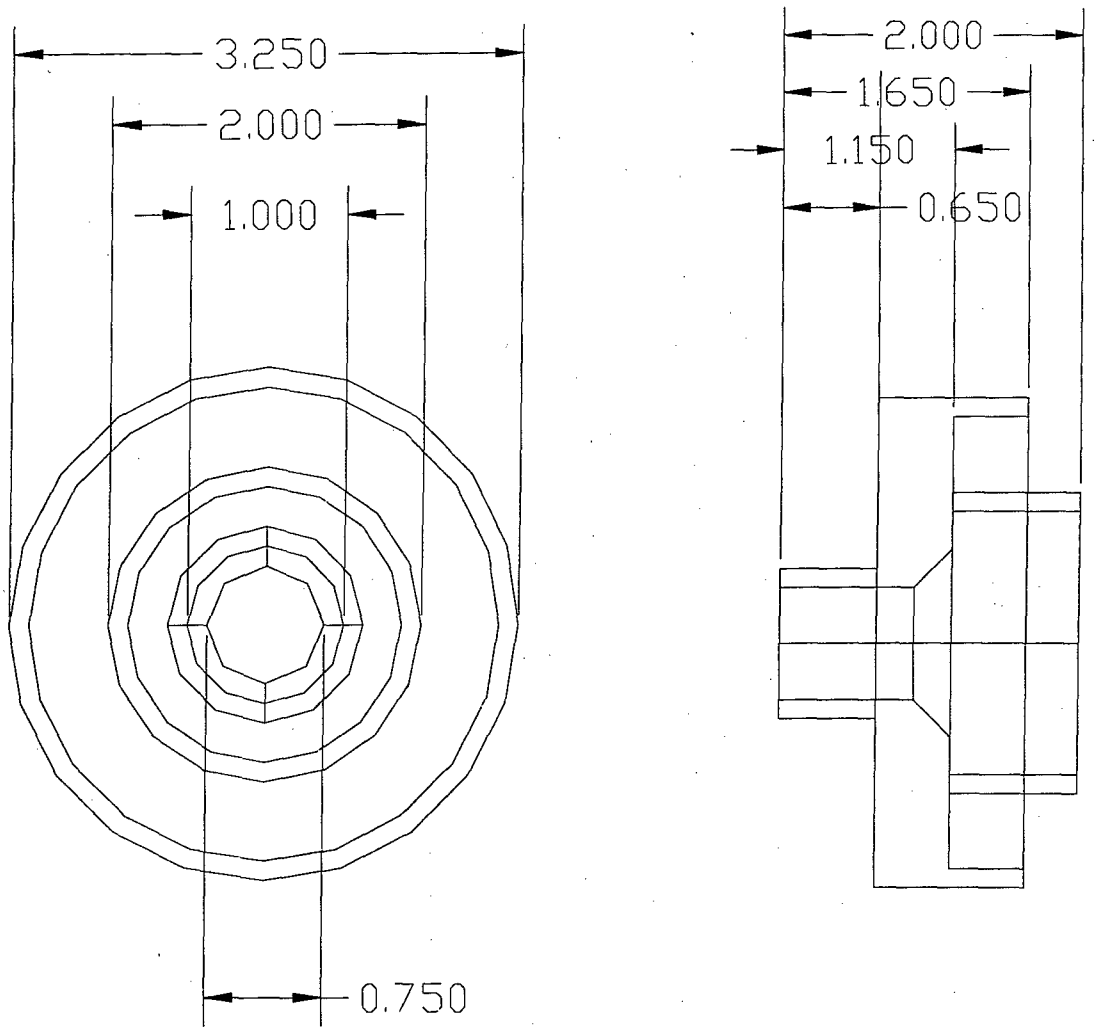

Figure 3a. Schematic drawing of PET nosepiece.
Note: dimensions are in inches, esophageal catheter not shown.

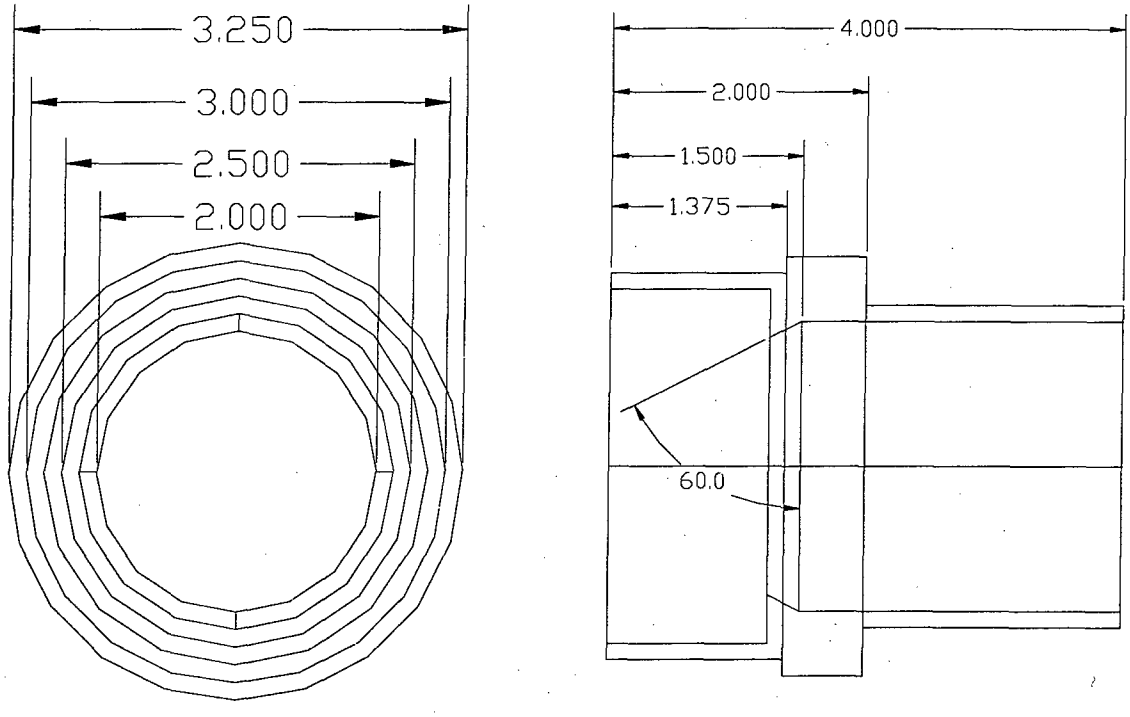

Figure 3b. Schematic drawing of PET thoraxpiece.
Note: dimensions are in inches, angles are in degrees.

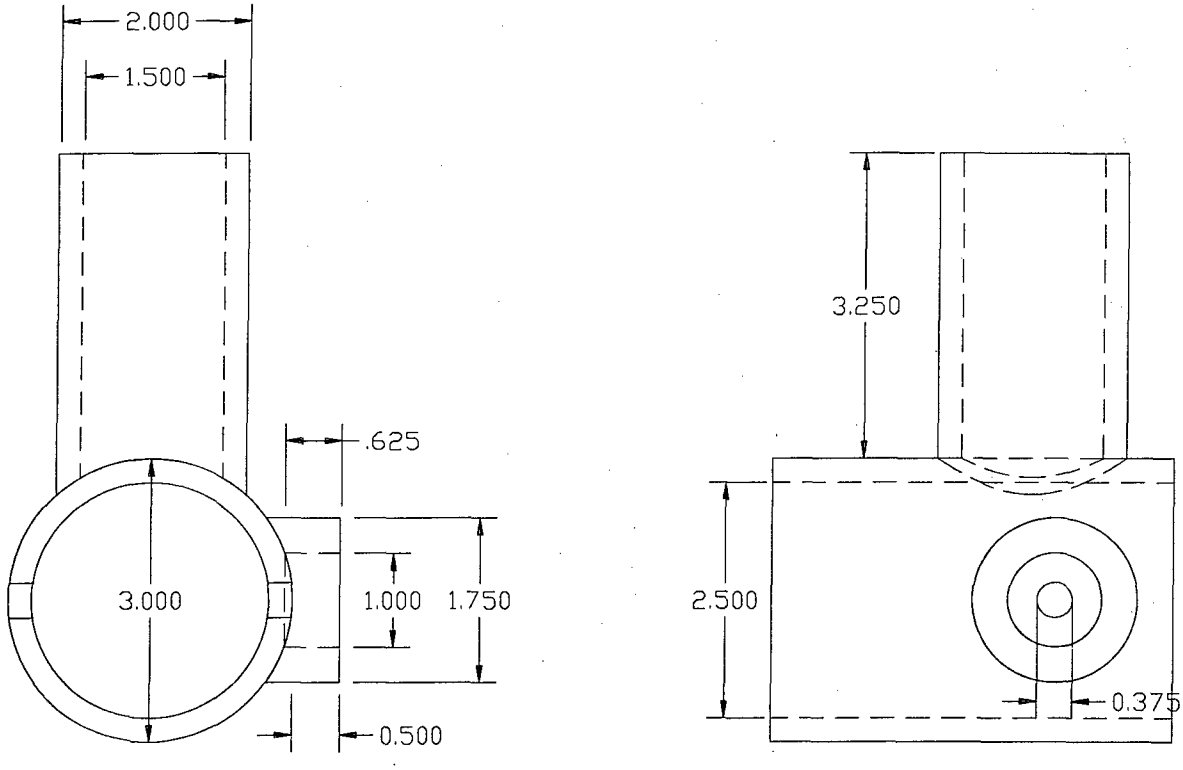

Figure 3c. Schematic drawing of PET main body.
Note: dimensions are in inches

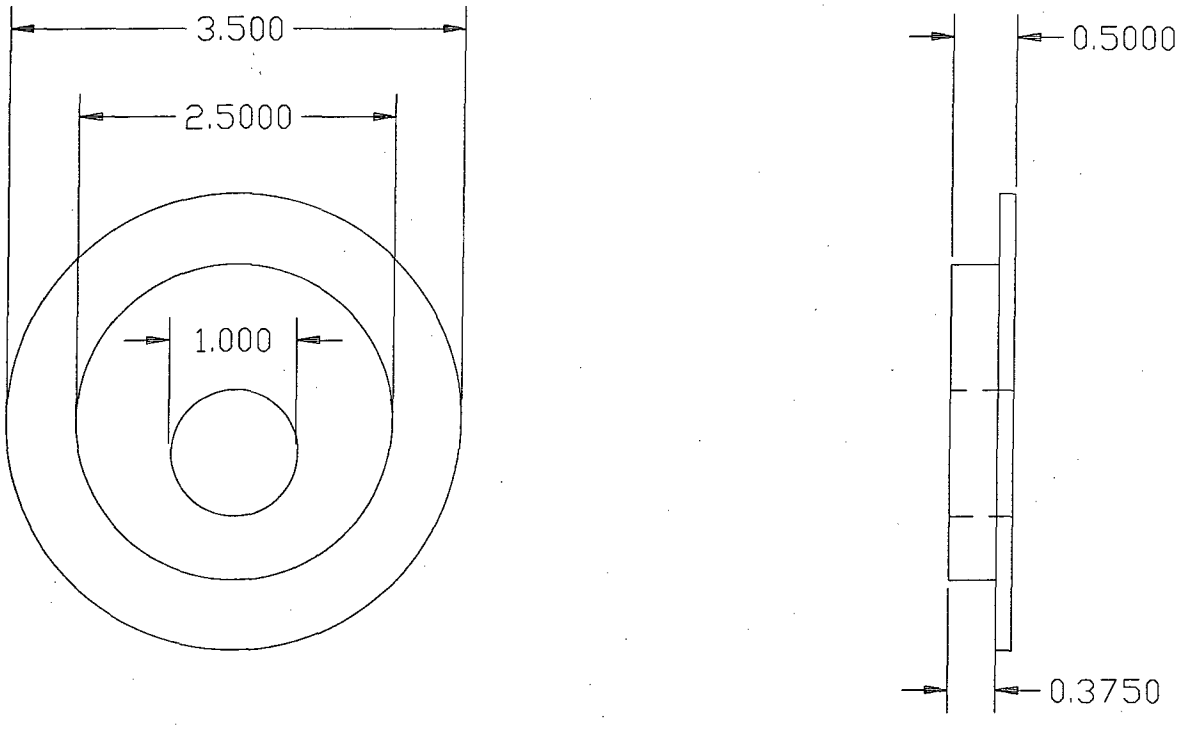

Figure 3d. Schematic drawing of PET tailpiece.
Note: dimensions are in inches, restraint plunger not shown.

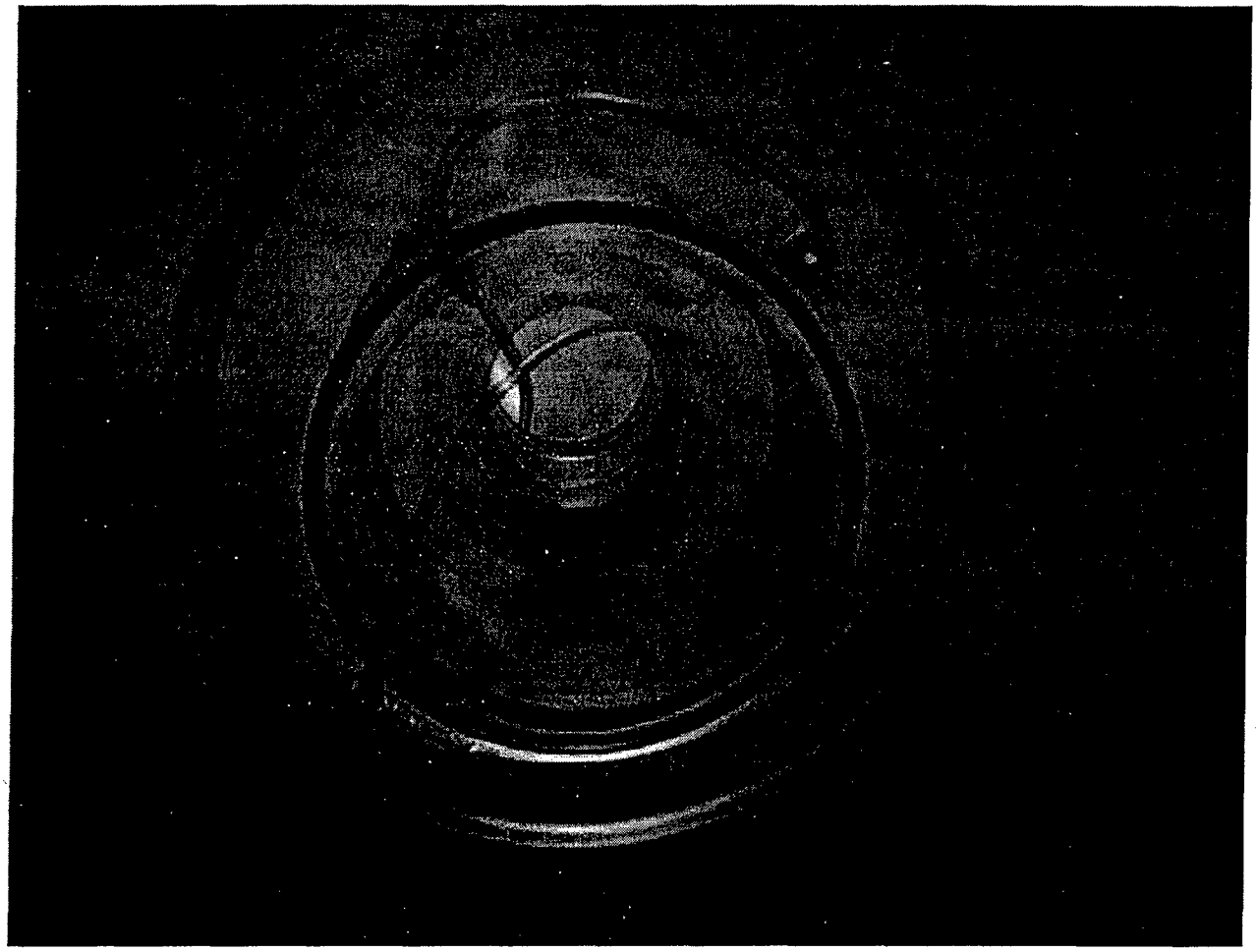

Figure 4. The PET nosepiece with esophageal catheter.

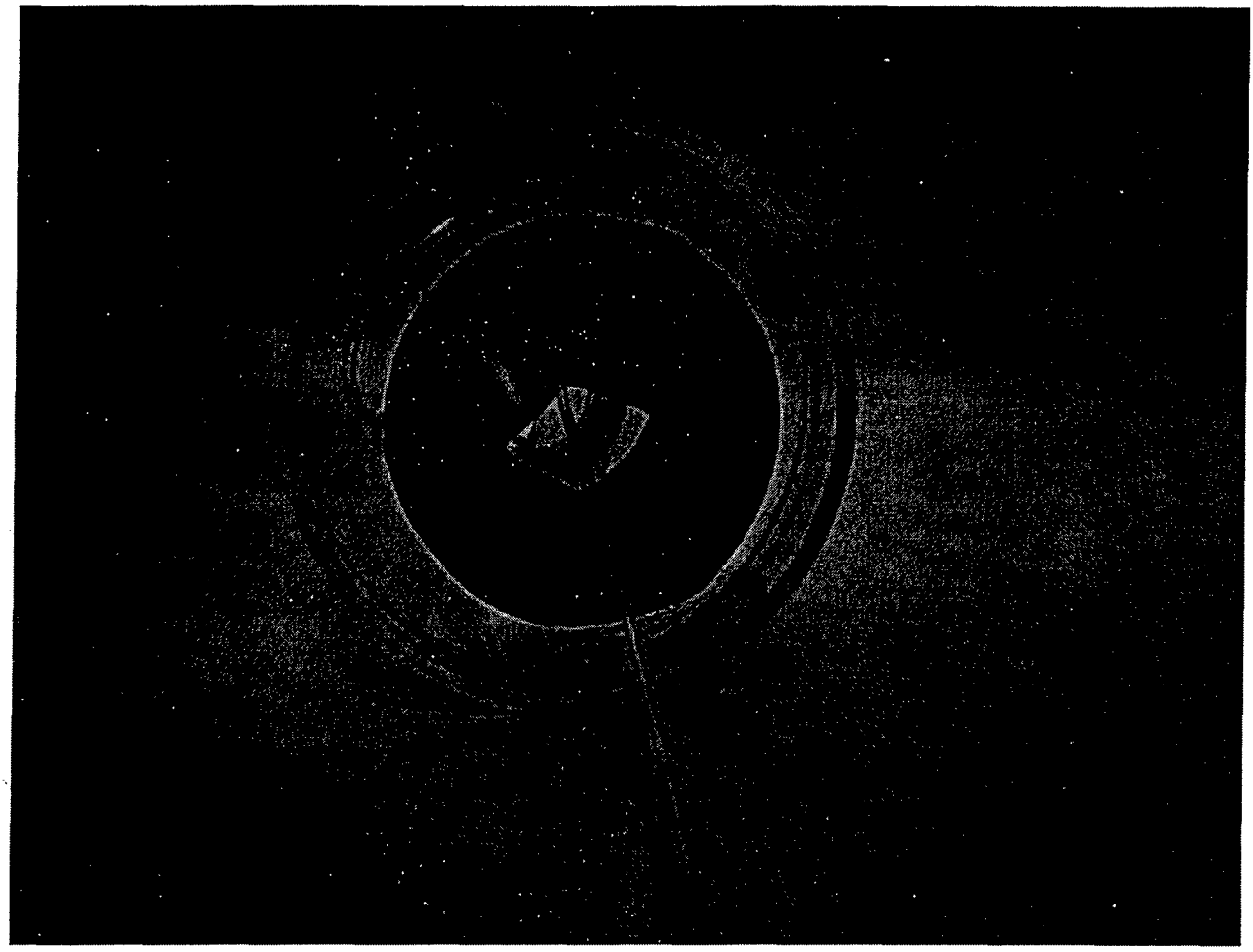

Figure 5. The PET nosepiece with latex membrane neck seal.

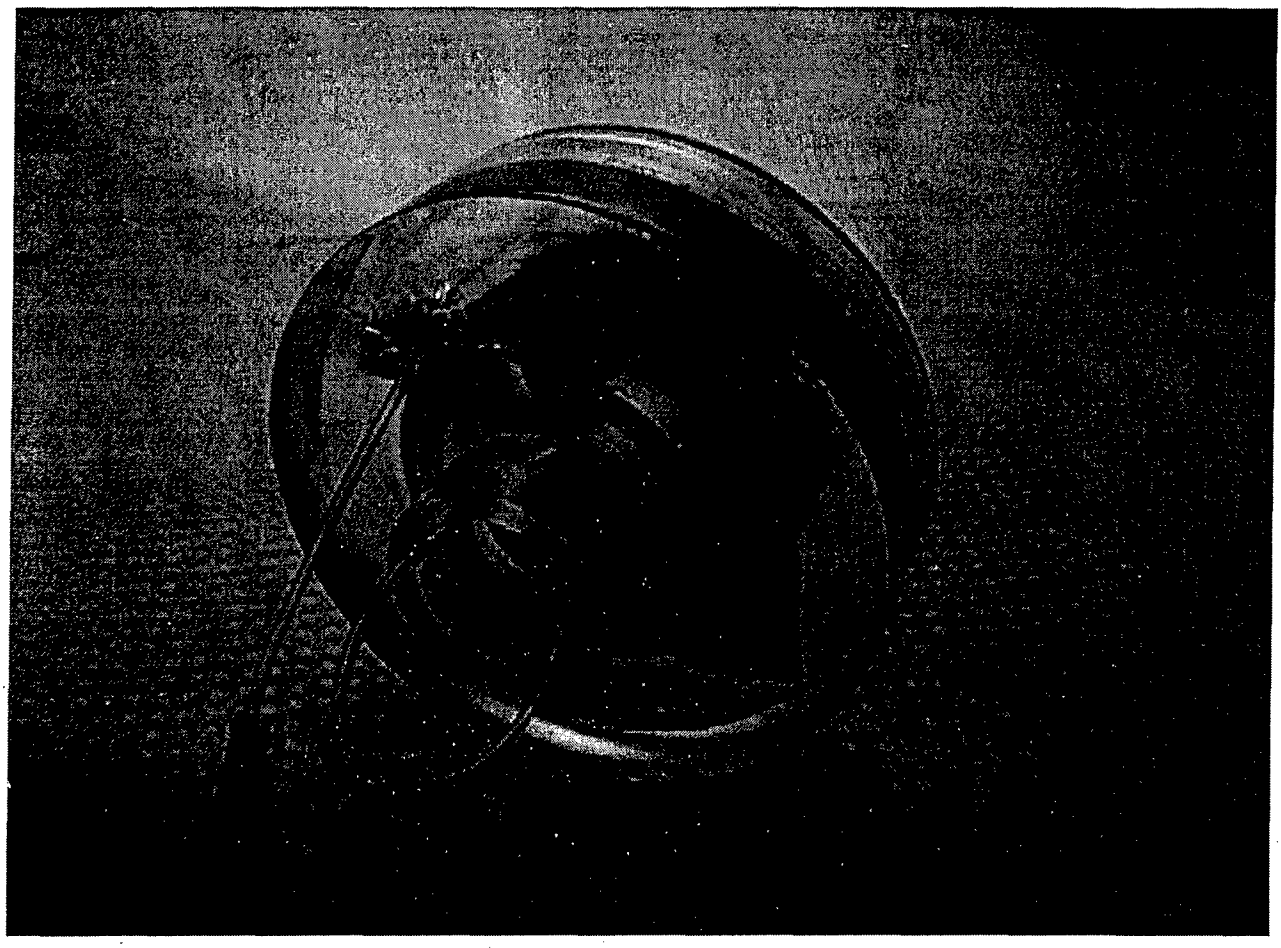

Figure 6. Esophageal catheter looped within the nosepiece/exposure chamber connector tip.

Figure 7. An animal inserted through the nosepiece through the neck seal.

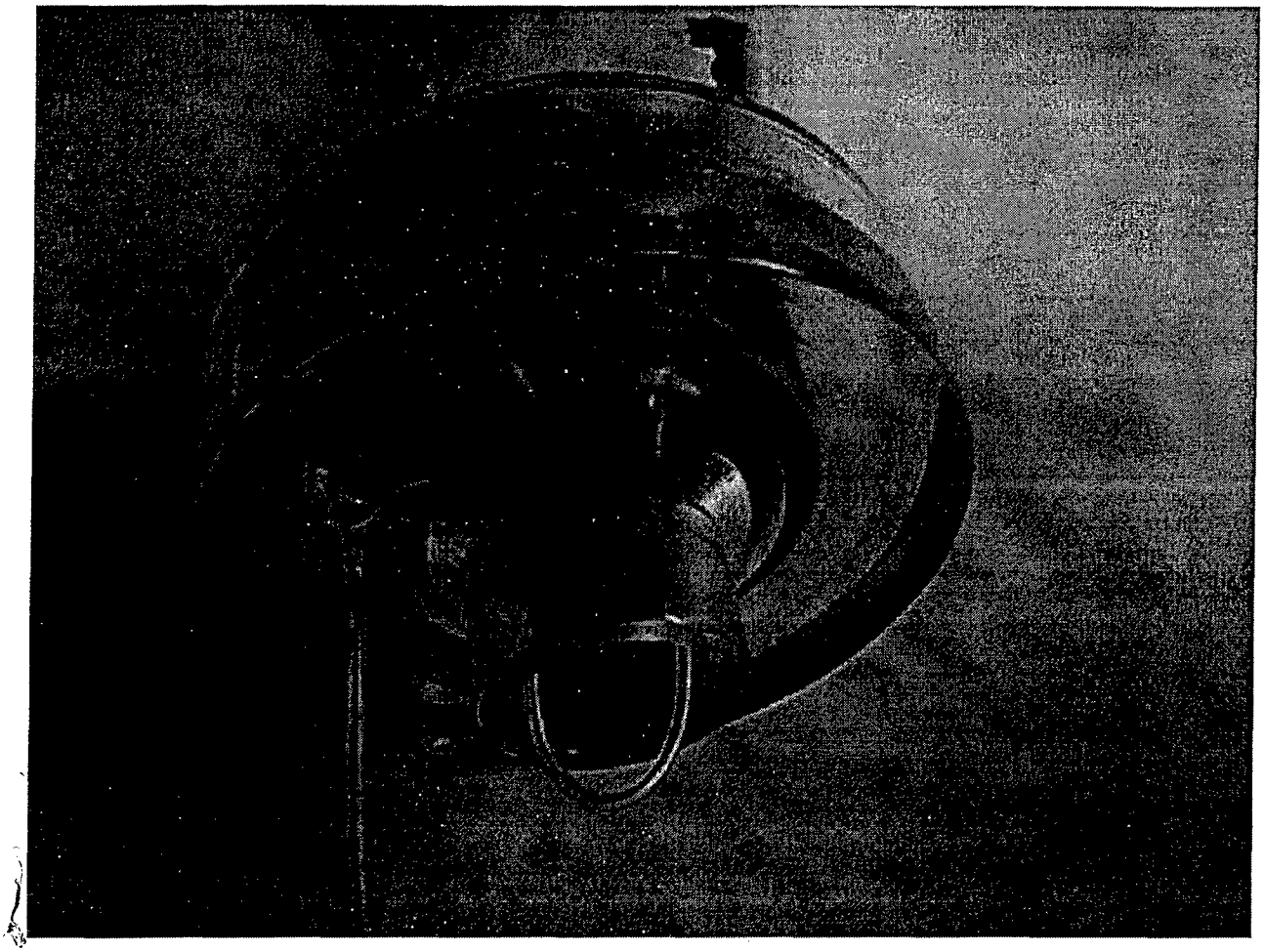

Figure 8. An animal with esophageal catheter inserted trans-orally.

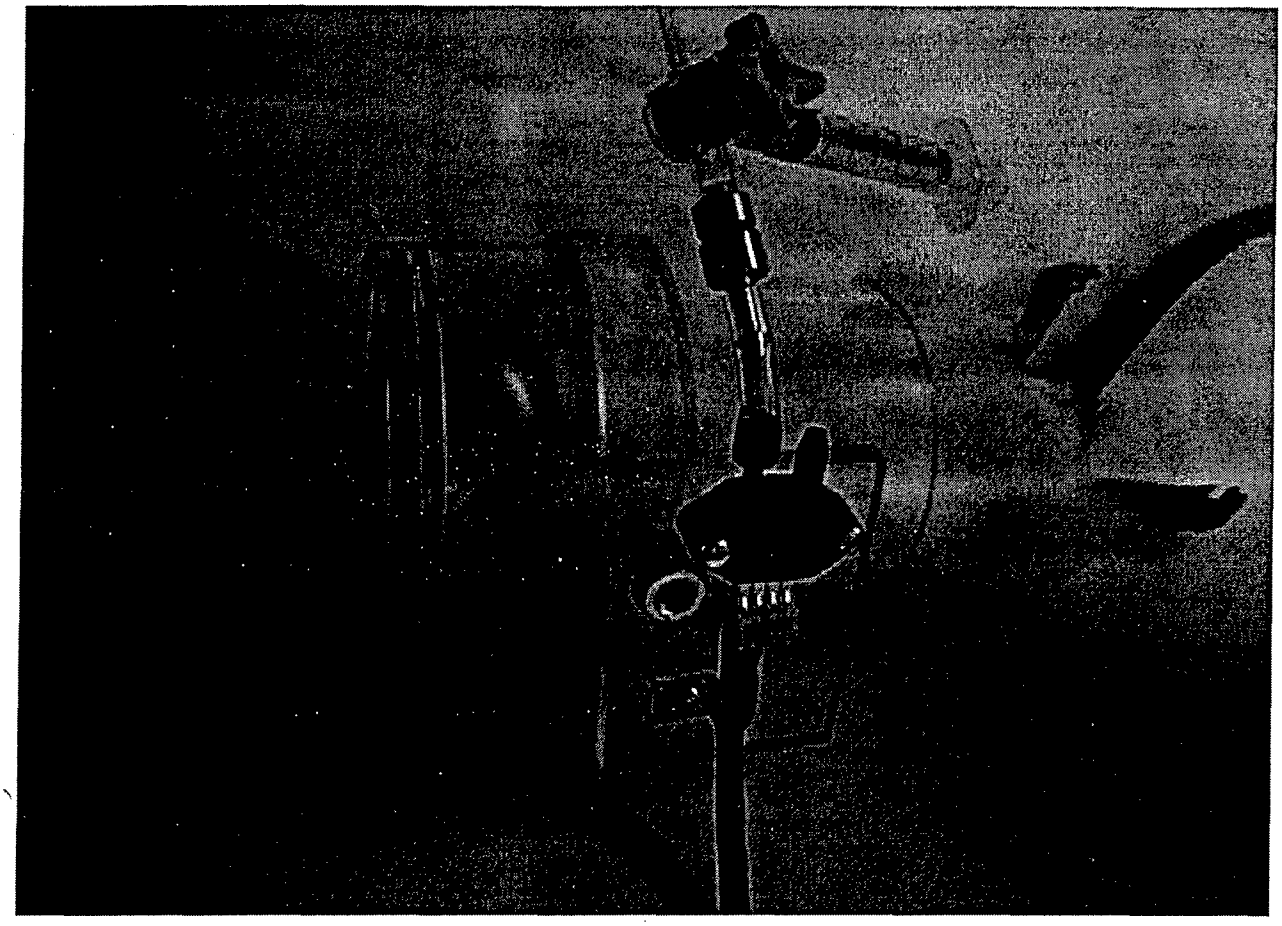

Figure 9. An animal placed in the PET thoraxpiece.
Note: placement of for paws forward of restraint ledge, this animal has been fully anesthetized for the photographic purposes.

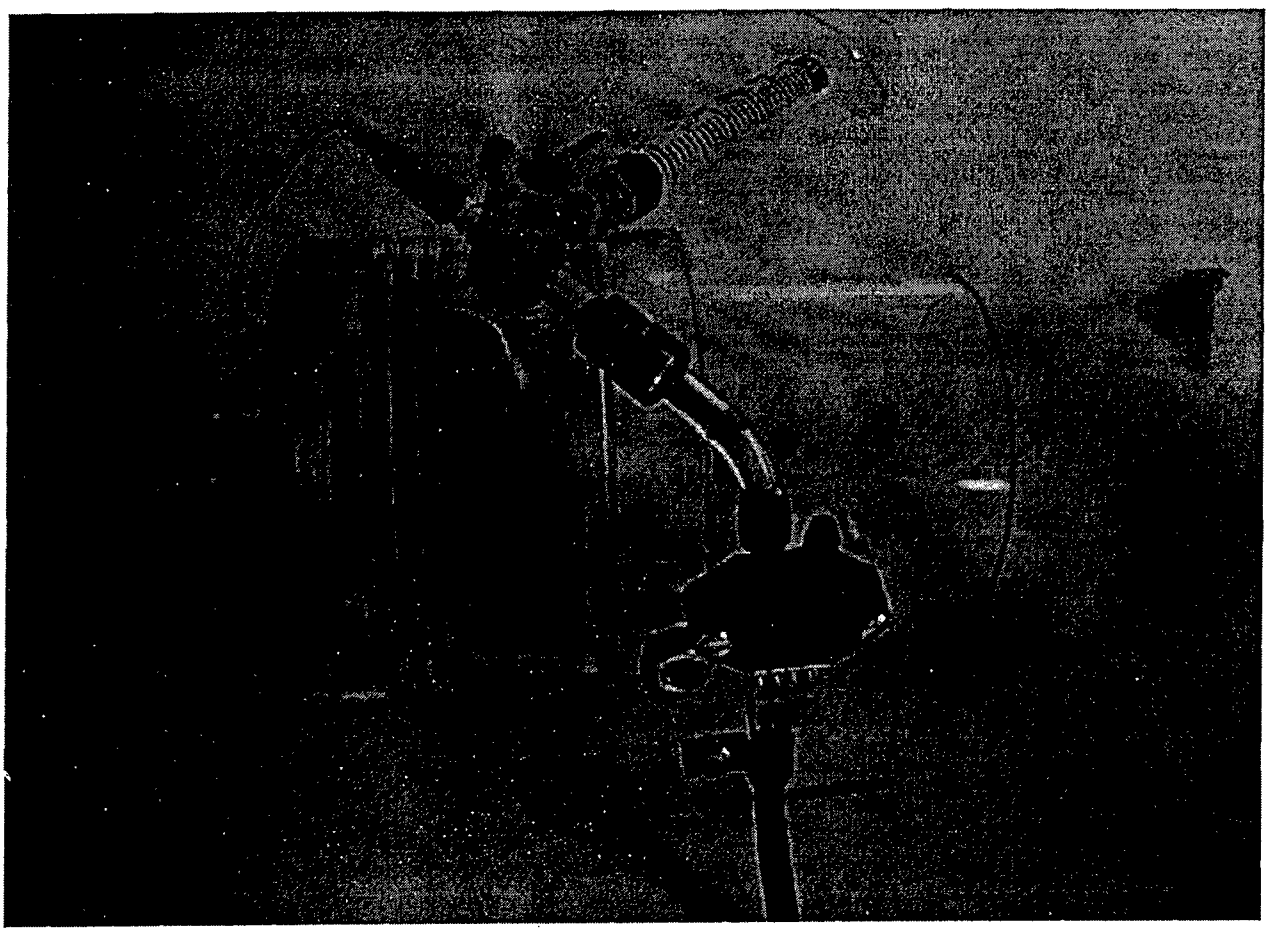

Figure 10. Connected nose and thoraxpieces.

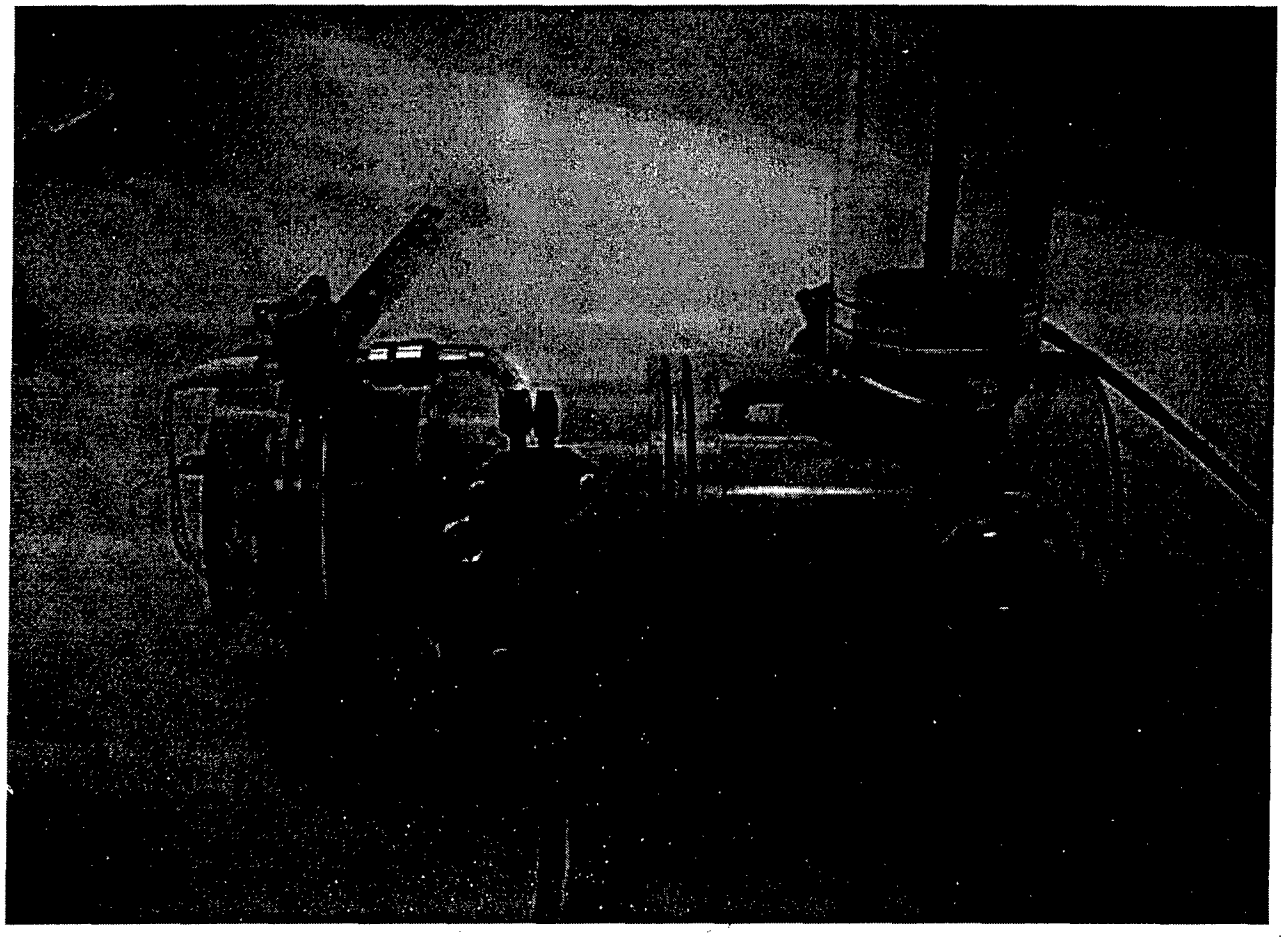

Figure 11. An animal in a fully assembled PET.

Note: tailpiece with restraint plunger now shown.

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Design of a Plethysmograph for the Measurement of Pulmonary Mechanics and Intrapleural Pressure in Small Animals during Exposure without Surgical Intervention

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13. **ABSTRACT** *(Maximum 200 words)* The real-time measurement of changes in respiratory mechanics, primarily dynamic compliance (C_{dyn}) and airway resistance (R_L), is often used to assess the pulmonary toxicity of inhaled materials and irritants thought to elicit an airway reactivity response. A simple volume displacement plethysmograph used for measurement of ventilation in spontaneously breathing rats was modified for the determination of C_{dyn} and R_L by including measurement of intrapleural pressure (Ppl). Accurate estimates of Ppl were obtained by measurement of esophageal pressure (Pes) using trans-oral insertion of a water filled catheter. Measurement of Pes did not require surgical intervention as is often required for measurement of Ppl directly. The use of conventional head-out plethysmography to measure ventilation and respiratory mechanics during exposure usually precludes the use of trans-oral insertion of an esophageal catheter to measure Pes. Thus, invasive methods must be used to measure Ppl. The combination head-out plethysmograph/nose-only exposure tube (PET), presently described, was found suitable for measurement of R_L and C_{dyn} using trans-oral catheterization for determination of Pes during exposure. Use of PET required did not require surgical intervention, did not obstruct the animal's normal breathing, and did not require extraordinary procedures for connection to a nose-only exposure chamber. Ventilation, breath waveform, and respiratory mechanics measurements in 36 Long Evans rats demonstrated that neither short-term restraint in the PET nor subsequent insertion of the esophageal catheter significantly altered ventilation or individual breath structure. R_L and C_{dyn} measured in normal rats using the PET did not differ from R_L and C_{dyn} determined using more conventional plethysmographic methods.

14. SUBJECT TERMS
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